Phenolic Composition of Bud Exudates of *Populus deltoides*

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Bud exudate of *Populus deltoides* clones originating from six central and eastern American states was examined by gas chromatography/mass spectrometry and the components were identified. The principal components of the bud exudate were the flavone galangin, the flavanone pinocembrin and the flavanonol pinobanksin, together with the related compounds pinocembrin chalcone, pinobanksin methyl ether and pinobanksin-3-acetate. The bud exudate composition was very different from that seen in a North American poplar of the section *Tacamahaca, P. balsamifera*.

The bud exudates from all *P. deltoides* specimens were similar excepting that from Vermont.

Introduction

The bud exudate of poplars is a complex mixture which usually consists of substituted benzoic acids and their esters, substituted phenolic acids and their esters, flavonoid aglycones, terpenoids and hydrocarbons [1–5]. We are assessing the possible application to the chemotaxonomy of the genus *Populus* of the analysis of bud exudate by gas-chromatography/mass spectrometry (GC/MS). We hope that such detailed analysis will assist in the taxonomy of this complex genus and may also permit identification of poplar clones which, although they are genetically different, cannot easily be distinguished morphologically. Positive identification of these poplar clones is necessary for their registration with the E.E.C. and bud exudate "fingerprints" may assist in this. The flavonoid aglycones of poplar bud exudate have already been used as a chemotaxonomic criterion [6] and preliminary results indicate that an analysis of the bud exudate of genetically different, but morphologically similar, clones of *P.* × *interamericana* Van Brockhuizen enables them to be correctly identified [7].

P. deltoides Marsh, the eastern cottonwood, has a wide distribution in central and eastern North America from the 50th to the 30th parallel [8]. Ecotypes occur within this extensive range and various of these have, at times, been described as distinct species, but they are usually regarded as subspecies [8–10]. *P. deltoides* is economically important, being a parent of the widely planted *P.* × *interamericana* clones (intersectional hybrids between *P. trichocarpa* Torr. and Gray and *P. deltoides*). We here report a detailed analysis using computer assisted GC/MS of bud exudate of *P. deltoides* obtained from clones originating from six central and eastern states of the U.S.A.

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Materials and Methods

Reagents

Bis-(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was obtained from Sigma (Poole, U.K.).

Plant material

The specimens of *P. deltoides* were derived from seed collected by Prof. Scott Pauley (formerly of Harvard University, U.S.A.) during the period 1950–1965 and subsequently grown at the Rijksstation voor Populierenteelt, Geraardsbergen, Belgium, or at the populetum of the Agricultural University of Wageningen, Netherlands (Table I).

Sample preparation

Exudate was collected by dipping 2–4 buds in 3 ml ethyl acetate in a screw-top conical glass tube for 10 s. The ethyl acetate was evaporated under a stream of N_2 and the extract freeze-dried for 10 min to remove residual water. After addition of 50 µl pyridine and 100 µl BSTFA, containing 1% TMCS, the tube was sealed and heated for 30 min

at 100 °C to produce trimethylsilyl (TMS) derivatives for gas chromatography.

Gas chromatography/mass spectrometry

As previously described [11] excepting that a 25 m × 0.32 mm ID Thames Chromatography (Maidenhead, U.K.) silica column coated with 0.5 μm of immobilized dimethylsiloxane was used, with a helium pressure of 76 kN/m^2.

Identification of compounds

Compounds in bud exudates were identified by comparison with GC retention times and mass spectra of reference compounds as described previously [11]. Flavonoid standards were either purchased from Apin Chemicals (Abingdon, U.K.) or from Plantech U.K. (Reading, U.K.), or provided as a gift by Professor E. Wollenweber (Darmstadt, F.R.G.). Other reference compounds were synthesized as described previously [1].

Chemical nomenclature

Common names are used in Table I and in the text. The chemical nomenclature of the basic compounds mentioned is as follows: alpinetin = 7-hydroxy-5-methoxyflavanone = pinocembrin-5-Me; alpinetin chalcone = 2′,4′-dihydroxy-6′-methoxychalcone; alpinone = 3,5-dihydroxy-7-methoxyflavanone = pinobanksin-7-Me; caffeic acid = *trans*-3(3,4-dihydroxyphenyl)-2-propenoic acid; chrysin = 5,7-dihydroxyflavone; cinnamic acid = *trans*-3-phenyl-2-propenoic acid; cinnamyl alcohol = *trans*-3-phenyl-2-propenol; *p*-coumaric acid = *trans*-3(4-hydroxyphenyl)-2-propenoic acid; coumaryl alcohol = *trans*-3(4-hydroxyphenyl)-2-propenol; ferulic acid = *trans*-3(3-methoxy-4-hydroxyphenyl)-2-propenoic acid; ferulyl alcohol = *trans*-3(3-methoxy-4-hydroxyphenyl)-2-propenol; galangin = 3,5,7-trihydroxyflavone; izalpinin = 3,5-dihydroxy-7-methoxyflavone = galangin-7-Me; kaempferol = 3,5,7,4′-tetrahydroxyflavone; naringenin = 5,7,4′-trihydroxyflavanone; pinobanksin = 3,5,7-trihydroxyflavanone; pinobanksin chalcone = 2′,4′,6′,α-tetrahydroxychalcone; pinocembrin = 5,7-dihydroxyflavanone; pinocembrin chalcone = 2′,4′,6′-trihydroxychalcone; quercetin = 3,5,7,3′,4′-pentahydroxyflavone; vanillic acid = 4-hydroxy-3-methoxybenzoic acid; vanillin = 4-hydroxy-3-methoxybenzaldehyde.

Results

The total ion chromatograms (TIC) of a typical bud exudate of *P. deltoides* from North Dakota and an atypical exudate from Vermont are shown in Fig. 1. Details from these chromatograms are shown in Fig. 2 with identified peaks numbered. The components of the bud exudate obtained from the plants listed in Table I are shown in Table II and the data is summarized in Table III. Only peaks exceeding 0.1% of the TIC are reported. Duplicate samples were analyzed to confirm the pattern of peaks in the chromatogram.

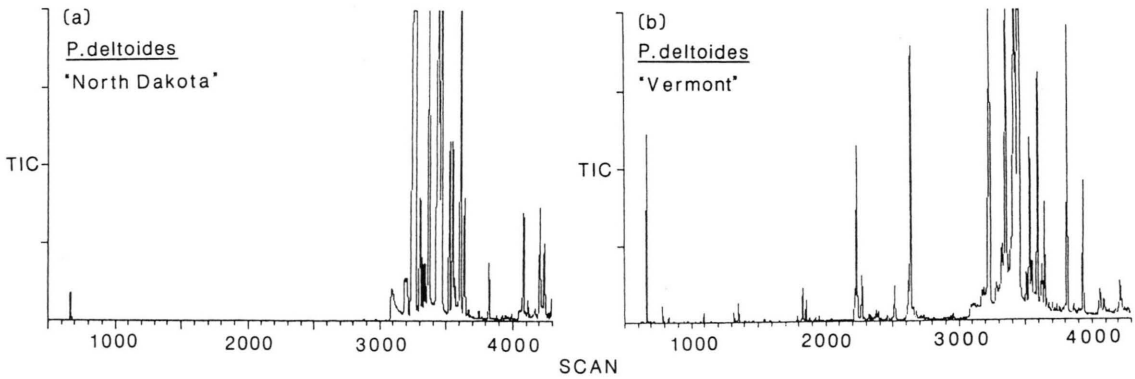

Fig. 1. Total ion chromatograms (TIC) of bud exudate, scans 500–4300 (MU 11–33), from (a) *Populus deltoides* "North Dakota", typical of the *P. deltoides* exudates studied and (b) *Populus deltoides* "Vermont", an atypical exudate.

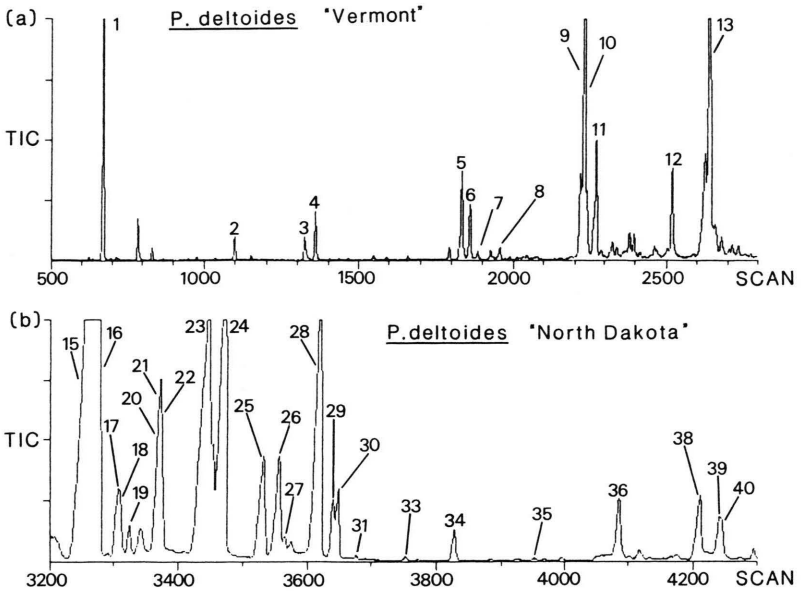

Fig. 2. (a) Total ion chromatogram (TIC) of bud exudate from *Populus deltoides* "Vermont", scans 500–2800 (MU 11–22). This region is atypical of the *P. deltoides* exudates analyzsed, having more compounds in the MU 11–22 region than do other exudates. (b) TIC of bud exudate from *Populus deltoides* "North Dakota", scans 3200–4300 (MU 24–33). This region is typical of the majority of the *P. deltoides* exudates analyzed. Identifications of numbered peaks are given in Table II.

Table I. Origin of North American *P. deltoides* specimens.

Specimen	Site of collection	Site of propagation	Reference Number
P. deltoides "Kansas"	Labette County	Wageningen	156
P. deltoides "North Dakota"	Morton County	Wageningen	172
P. deltoides "Vermont"[1]	Bennington County	Wageningen	1078
P. deltoides "Wisconsin"	Dane County	Geraardsbergen	572
P. deltoides "Missouri" (A)	Boone County	Geraardsbergen	570
P. deltoides "Missouri" (B)	St. Charles County	Geraardsbergen	573
P. deltoides "Conneticut"	County not known	Geraardsbergen	583

[1] "Vermont" is a cross made in 1961 between Scott-Pauley Ref. Nos. 344 and 354 collected in Bennington County, Vermont.

Table III. Summary of the major constituents of *Populus deltoides* bud exudate.

	% Total ion current[1] *P. deltoides*						
	Vermont	Conneticut	Wisconsin	Missouri A	Missouri B	Kansas	North Dakota
Substituted benzoic acids and their esters	3	–	–	–	–	T[2]	1
Substituted phenylpropenoic acids and their esters	20	3	T	3	5	6	10
Chalcones[3]	6	8	23	19	18	19	15
Dihydrochalcones	–	–	–	–	–	–	–
Flavones	10	36	16	24	19	18	19
Flavanones[4]	46	48	56	54	53	51	51
Terpenoids	3	–	–	T	1	1	–

[1] The ion current generated depends on the characteristics of the compound concerned and is not a true quantitation [1].
[2] T (trace) indicates amounts of between 0.1% and 1%. Compounds marked – comprised either <0.1% of the TIC. or were not detected.
[3] This could be an overestimate as some flavanones may partially convert to the corresponding chalcones during sample preparation and/or analysis [1].
[4] The various esters of short chain acids and benzoic acid with pinobanksin are included here.

Table II. Composition of bud exudate of *Populus deltoides*. Peak numbers correspond to those given in chromatograms shown in Fig. 2. GC retention times given in methylene units (MU)[1] are given to two decimal places to indicate the elution sequence of peaks which chromatograph closely. Factors such as concentration of the compound concerned, together with the characteristics of a particular GC column are liable to affect the chromatography and for general purposes the MU figures are probably reliable to only a single decimal place.

Peak No.	Compound	No. TMS Groups	MU	% Total ion current[2] *P. deltoides* Vermont	Connecticut	Wisconsin	Missouri A	Missouri B	Kansas	North Dakot
1	benzoic acid	1	12.32	3	–	–	–	–	T[3]	T
2	cinnamyl alcohol[4]	1	14.06	T	–	–	–	–	–	–
3	vanillin	1	15.00	T	–	–	–	–	–	–
4	cinnamic acid	1	15.17	T	–	–	–	–	–	–
5	sesquiterpene alcohol	1	17.27	1	–	–	T	T	T	–
6	sesquiterpene alcohol	1	17.39	1	–	–	–	T	T	–
7	vanillic acid	2	17.51	T	–	–	–	–	–	–
8	*p*-coumaryl alcohol	2	17.83	T	–	–	–	–	–	–
9	*p*-coumaric acid	2	19.29	5	–	T	–	T	–	–
10	ferulyl alcohol	2	19.32	2	–	–	–	–	–	–
11	sesquiterpene alcohol	1	19.50	1	–	–	–	T	T	–
12	ferulic acid	2	20.77	1	–	–	–	–	–	–
13	caffeic acid	3	21.44	6	–	T	T	–	T	–
14	benzyl *p*-coumarate	1	22.71	1	–	–	–	–	–	1
15	pinocembrin	2	24.92	7	15	30	26	22	20	19
16	pinocembrin chalcone[5]	3	24.99	4	8	21	18	17	15	14
17	alpinetin	1	25.25	–	–	3	2	3	1	3
18	alpinetin chalcone[5]	2	25.28	T	T	T	–	T	2	1
19	phenylethyl *p*-coumarate	1	25.45	–	1	–	T	2	1	1
20	pinobanksin-3-acetate chalcone[5]	3	25.67	2	–	2	1	1	2	T
21	pinobanksin	3	25.77	3	4	3	8	7	6	5
22	benzoyloxy compound[6]	1	25.79	5	T	T	–	T	T	2
23	pinobanksin methyl ether[7]	2	26.16	11	8	5	4	8	9	11
24	pinobanksin-3-acetate	2	26.34	25	20	14	14	13	15	11
25	benzyl caffeate	2	26.79	1	–	–	–	–	T	4
26	chrysin	2	27.04	4	8	3	6	4	6	3
27	galangin methyl ether[8]	2	27.08	T	1	T	1	1	T	T
28	galangin	3	27.45	5	23	10	16	11	10	11
29	phenylethyl caffeate	2	27.65	1	2	–	–	1	1	2
30	dihydroxymonomethoxyflavone	2	27.68	1	4	2	T	1	1	1
31	cinnamyl *p*-coumarate	2	27.82	2	–	T	2	1	1	1
32	pinobanksin-3-(iso)butanoate[9]	2	28.00	–	T	–	–	–	–	–
33	naringenin	3	28.42	–	T	T	–	–	–	–
34	C 29 hydrocarbon	–	29.00	6	2	T	T	1	3	1
35	cinnamyl caffeate	2	29.96	3	–	–	T	T	2	–
36	kaempferol	4	30.94	T	T	1	1	2	1	2
37	kaempferol-3-methyl ether	3	30.96	–	–	–	T	T	–	–
38	pinobanksin-3-benzoate	2	31.99	–	–	–	–	–	–	2
39	quercetin methyl ether	4	32.24	T	–	–	–	–	–	1
40	quercetin	5	32.26	T	–	T	T	T	–	1

[1] Methylene units are defined by Dalgliesh *et al.* [17].
[2] The ion current generated depends on the characteristics of the compound concerned and is not a true quantitation [1].
[3] T (trace) indicates amounts of between 0.1% and 1%. Compounds marked – comprised either $<0.1\%$ of the TIC or were not detected.
[4] The chemical nomenclature is given in Methods.
[5] This could be an overestimate, as some flavanones may partially convert to the corresponding chalcones during sample preparation and/or analysis [1].
[6] Probably the mono-TMS of the benzoyl ester of ferulyl alcohol.
[7] Probably pinobanksin-5-methyl ether (see section on novel compounds and Fig. 3 b).
[8] Our standards of galangin-3-methyl ether and izalpinin (galangin-7-methyl ether) co-chromatograph and have very similar spectra. This could be either one, or a mixture of both, compounds.
[9] We do not know whether the substituent at the 3 position is linear or branched.

The principal components of bud exudate of *P. deltoides* are the flavone galangin[28]+ = 3,5,7-trihydroxyflavone and the flavanones pinocembrin[15] = 5,7-dihydroxyflavanone and pinobanksin[21] = 3,5,7-trihydroxyflavanone (more conventionally a flavanonol), together with the related compounds pinocembrin chalcone[16], pinobanksin methyl ether[23] and pinobanksin-3-acetate[24]. The flavanones and chalcones comprise 52–79% of *P. deltoides* bud exudate (Table III). Wollenweber [6] records two flavonoids from *P. deltoides* bud exudate which are not listed in Table II. Of these quercetin-7,3′-dimethyl ether is present [MU 31.62] but represents less than 0.1% of TIC and is therefore not listed. We do not have a standard of the second, quercetin-3,3′-dimethyl ether, but if present it would again represent less than 0.1% of TIC. We wish to emphasize that the TIC recorded seriously underestimates the occurrence in bud exudate of the higher molecular weight polyhydroxyflavonoids, such as quercetin[40] = 3,5,7,3′,4′-pentahydroxyflavone and its monomethyl[39] and dimethyl ethers. This is because these compounds do not chromatograph as well as do the smaller flavonoids [1].

The bud exudate of *P. deltoides* "Vermont" is distinct from that of all the other exudates in having 3% benzoic acid[1], 5% *p*-coumaric acid[9] and 6% caffeic acid[13]. These acids are present in only trace quantities in the other exudates (Table II). *P. deltoides* "Vermont" is also distinctive from other *P. deltoides* specimens in having lower levels of pinocembrin and galangin but higher levels of pinobanksin-3-acetate in its bud exudate (Table II).

+ Superscripts refer throughout to peak numbers in Fig. 2 and Table II.

Novel compounds

We have recently described a series of pinobanksin derivatives esterified in the 3 position with various aliphatic acids in *P. fremontii* S. Wats. and *P. maximowiczii* Henry [12]. Of these an ester of pinobanksin with butanoic acid[32] or isobutanoic acid is present in sufficient quantity to record in Table II. In bud exudate of *P. deltoides* "North Dakota" we also identify pinobanksin esterified in the 3 position with benzoic acid. The mass spectrum of this novel compound, pinobanksin-3-benzoate[38] = 5,7-dihydroxy-3-benzoyloxyflavanone, is shown as its bis-TMS derivative in Fig. 3a. The fragmentation follows the same pattern as that described for other trimethylsilylated pinobanksin esters [12]. Characteristic ions are $[M-15]^+$ m/z = 505, composition M−[CH_3 from TMS]; $[M-119]^+$ m/z = 401, composition M−[CH_3; COC_6H_4]; $[M-137]^+$ m/z = 383, composition M−[CH_3; H; $OCOC_6H_5$]. The ions at m/z = 105, composition COC_6H_5, and at m/z = 77, composition C_6H_5, confirm the benzoyl ester.

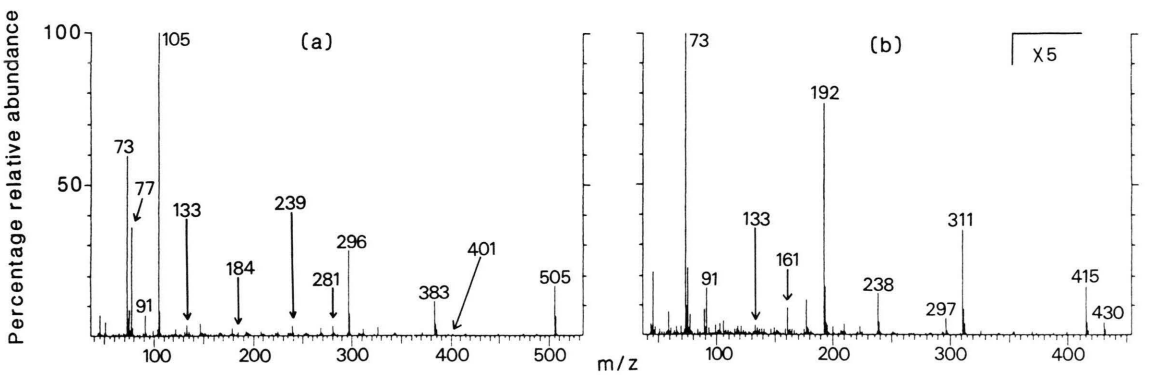

Fig. 3. (a) Mass spectrum recorded at 70 eV of pinobanksin-3-benzoate bis-TMS[38] = 5,7-dihydroxy-3-benzoyloxyflavanone bis-TMS, $[M]^+$ m/z = 520; (b) mass spectrum recorded at 70 eV of a dihydroxymonomethoxyflavanone bis-TMS[23], probably pinobanksin-5-methyl ether bis-TMS = 3,7-dihydroxy-5-methoxyflavanone bis-TMS (see section on novel compounds). The spectra shown are both from bud exudate of *Populus deltoides* "North Dakota".

This is the first report of a flavanone esterified with benzoic acid, although the flavone 7-benzoyl chrysin = 5-hydroxy-7-benzoyloxyflavone has been previously reported from *Baccharis* [13].

There is a further benzoyl compound[22] which is believed to be the benzoyl ester of *trans*-ferulyl alcohol, present as its mono-TMS derivative $[M]^+$ m/z = 356. This compound chromatographs close to pinobanksin[21]. An uncontaminated spectrum has not so far been obtained nor are we able to synthesize the compound to confirm its identity since we do not have the ferulyl alcohol necessary for the synthesis.

Analysis of the mass spectrum of the dihydroxy monomethoxyflavanone[23] which is present in all bud exudates analyzed here (Table II) indicates it to be either pinobanksin-5-methyl ether or pinobanksin-7-methyl ether (alpinone). The 7-methyl ether has been identified in *Alpina* and *Pinus* [14] and the 5-methyl ether has been identified in propolis [15]. It is known that European propolis frequently incorporates poplar bud exudate [1, 4, 5] and we therefore suggest the compound is pinobanksin-5-methyl ether = 3,7-dihydroxy-5-methoxyflavanone. The mass spectrum of the bis-TMS derivative of this compound is shown in Fig. 3b.

Discussion

The analyses of the *P. deltoides* specimens indicate that these North American poplars of the Section *Aigeiros* have bud exudate which is predominantly composed of flavanones, chalcones and esters of flavanones together with the flavone galangin. One closely related group of compounds, pinocembrin, pinobanksin and their immediate derivatives constitute the bulk of the bud exudate. This contrasts sharply with the situation found in the North American balsam poplar *P. balsamifera* L. (= *P. tacamahaca* Mill.) of the Section *Tacamahaca* [3]. Here flavanones form less than 3% of the total bud exudate and dihydrochalcones, which are essentially absent from poplars of the Section *Aigeiros* analyzed, form 40% or more of the total exudate.

P. deltoides is distributed over much of central and eastern North America. It is not possible to draw extensive conclusions concerning variation in chemotaxonomy within the species from the limited data presented here. It is noticeable however that the bud exudate of the specimen from Vermont is distinctive, and, if typical of trees in that area, indicates that these poplars represent a chemotaxonomically distinct race of the *P. deltoides*.

We recently noted that bud exudate of *P. candicans* Ait. (*P. gileadensis* Rouleau) closely resembled that of *P. balsamifera* and suggested that *P. candicans* was indeed a clone of *P. balsamifera* rather than a cross between *P. balsamifera* and *P. deltoides* [16]. The analyses reported here, which demonstrate bud exudate of *P. deltoides* to be markedly different from that of both *P. balsamifera* and *P. candicans*, support that conclusion.

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